

# HiGro User Procedure

- **Important:** Training is **REQUIRED** before usage. Schedule training with a member of the Microarray/Genomics Core Facility.
- You must sign up in advance.
- You must submit an email to the Microarray Facility that you are submitting cultures and fill out the EST sequencing Excel spreadsheet located in Microarray Facility inbox.

## Culture Plate Conditions

- Fill wells with 500 $\mu$ L (for 96-well) or 120 $\mu$ L (384-well, you need to use the Biomek robot to dispense the media, please ask the Genomics facility technicians for help) of TB + Salts and appropriate antibiotic.
  - **Terrific Broth (TB)**
    - 12 g Bacto-tryptone
    - 24 g yeast extract
    - 4 ml glycerol
    - Add Milli-Q H<sub>2</sub>O to total 900 ml, and autoclave.
  - **10X TB salts:**
    - 2.31 g KH<sub>2</sub>PO<sub>4</sub>
    - 12.54 g K<sub>2</sub>HPO<sub>4</sub> (potassium phosphate, dibasic)
    - Add Milli-Q H<sub>2</sub>O to total 100ml
  - Autoclave, cool and add to Terrific Broth along with selective antibiotic.
- Plug the oxygen regulator into the wall receptacle.
- Turn on the oxygen bottle.
- Fill oxygen water bottle with 500 ml of MilliQ water.
- Turn on HiGro shaker and allow chambers to warm.
- Place plates into HiGro cassettes and place the cassettes into the HiGro chamber.
- Tighten the cassette support knob (do not over tighten cassette support knobs).
- Incubate for 16 hours (96-well) or 20 hours (384-well) at 37°C, shaking at 550 rpm (Do not seal the plate).
- **Note:** 20 blocks is the maximum capacity, be sure to place an empty block in the top slot of the cassette to catch condensation.
- **Make Sure To Turn Off Oxygen Bottle And Unplug The Regulator.**